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Cop. 2 A Summary of Current Program 7/1/65  
and Preliminary Report of Progress  
for 7/1/64 to 6/30/65

HUMAN NUTRITION RESEARCH DIVISION  
of the  
AGRICULTURAL RESEARCH SERVICE  
UNITED STATES DEPARTMENT OF AGRICULTURE  
and related work of the  
STATE AGRICULTURAL EXPERIMENT STATIONS

This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1964, and June 30, 1965. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Human Nutrition Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

UNITED STATES DEPARTMENT OF AGRICULTURE

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## INTRODUCTION

The research reported here presents recent progress in understanding the nutritional needs of normal man and the manner by which these needs can best be met by food. The research involves studies of the absorption, transport, and metabolism of individual nutrients in the body as related to age, activity, and environmental conditions. Studies of metabolic processes and nutritional requirements in man are preceded, guided, and expedited by results from intensive studies on laboratory animals and lower forms of life in which more factors can be controlled and physiological responses can be measured during each stage in the life cycle and during successive generations. The research includes the nutritive and other consumer values of foods as measured by chemical or physical means and by biologic response, and the effects of household practices upon the nutritive value and inherent qualities of foods. Knowledge gained from human nutrition research can be used to influence the food habits and improve the nutritional status of man. It also can influence market demand and in turn the orientation of production of agricultural products.

The program is carried on by the Human Nutrition Division of the Agricultural Research Service of the U. S. Department of Agriculture. It is conducted at the Agricultural Research Center near Beltsville, Maryland, and under contract and cooperative agreement with universities, medical schools, hospitals, and industry. In addition, the Division collaborates with Regional programs of the State Experiment Stations. The Federal scientific effort devoted to this research in Fiscal Year 1965 totalled 69.2 professional man-years with 60.4 engaged in the program near Beltsville, Maryland, and the equivalent of 8.8 in contract and cooperative agreements. The program is divided among study of:

	Intramural	Extramural	Total
Functions and metabolism of nutrients	19.8	2.7	22.5
Human metabolism and requirements for nutrients	5.9	1.8	7.7
Nutrient value of foods	18.6	3.1	21.7
Other food qualities and consumer use	16.1	1.2	17.3

Basic information on human nutrition is needed for conservation and optimal utilization of human and food resources and for nutritional well-being of the population. The Division has contributed to this goal by providing information on nutritional and food needs and on the qualities of foods which influence their usefulness to consumers. Some of these contributions have been summarized here:

Regional and seasonal variations in nutrients of market basket diet.

ARS scientists found considerable variation in the sucrose content of a representative diet prepared from foods purchased in different areas of the United States and in different seasons. The highest sucrose level was found in foods purchased in the springtime and in Washington, D. C. Variations in other carbohydrate fractions, mineral elements, total fat, and nitrogen were not large enough to be considered nutritionally important, though there was considerable variation in the aluminum content.

Data on the nutrient composition, including 10 mineral elements and 5 carbohydrate fractions, were obtained from analyses of 20 composites of foods prepared for a 14-day diet for 16-19 year old boys. The combinations of foods were selected as representative of a good diet for teenage youngsters, based on results of food consumption surveys and the ARS "moderate income plan" for nutritionally adequate diets for 16- to 19-year old boys. This age group was selected for the prototype diet because it eats more food than any other age group.

Foods for the diet were market-purchased in 5 consecutive seasonal quarters, from 4 chain stores in the Washington, D. C. area, and from St. Louis, Missouri, and San Francisco, California.

Maternal intake of vitamin B<sub>1</sub> important to development of healthy offspring. That consumption of an adequate diet during pregnancy may fail to correct completely for dietary inadequacies prior to pregnancy in the rat was shown in recent research by nutrition specialists. An adequate diet prior to pregnancy also failed to protect the rat from dietary inadequacies during pregnancy. Fewer offspring, and abnormally high levels of the blood protein gamma globulin resulted when rats were subjected to a prolonged partial deficiency of thiamine (vitamin B<sub>1</sub>) prior to pregnancy, in spite of an adequate nutrient intake during pregnancy. Abnormally high levels of gamma globulin may suggest the rallying of the body to a danger signal in metabolism. Small offspring, low thiamine content in the liver, and abnormally high values of gamma globulin in the blood of the mother were observed when thiamine intake was inadequate during pregnancy, even though the prepregnancy diet was adequate. The findings further support the importance of adequate nutrition prior to and during pregnancy to assure normal development of healthy young.



Evidence of nutritional significance of individual carbohydrates. Further evidence has been obtained by ARS scientists that the metabolic response to carbohydrates may vary depending on other components in the diet and upon the inherited characteristics of the individual. For some individuals carbohydrates, often considered chiefly as a source of calories, may play a significant role in controlling various metabolic processes, including those related to the deposition of fat and cholesterol in body tissues. One strain of rats fed an experimental diet considered to be nutritionally adequate and containing sucrose as the only carbohydrate had extremely high levels of cholesterol in the blood and large amounts of fat and cholesterol in the liver. When the dietary carbohydrate was corn starch or glucose lower fat and cholesterol levels were observed. With rats of different heredity fed these same diets cholesterol and fat levels were low and the effect of dietary carbohydrate was of questionable significance.

Protein constituents affect energy production in cells. Recent research by ARS scientists suggests that an excess of the amino acid serine (a building block of protein) or one of its metabolic products may interfere with the formation of a key compound in the conversion of foods to energy in a single-cell animal. High levels of serine inhibited growth and altered the pattern and concentration of free amino acids in the organism. Addition of other amino acids, particularly alanine, arginine, aspartic acid, or glutamic acid reversed the growth inhibition and restored the normal pattern and concentration of free amino acids in the cell. These findings give insight into why nutrition may be impaired if dietary proteins and supplements provide amino acid patterns that differ markedly from requirements.

Some pesticides influence characteristics of food flavor. That flavor and eating quality of both meat and plant products may be affected by the use of pesticides during production has been shown by ARS food specialists in cooperation with other scientists. Rib cuts from beef animals sprayed with a pesticide used to control cattle grubs and horn flies had more off-flavor than those from untreated animals; the flavor of ground round, liver and kidney was not adversely affected. Off-flavors in potatoes grown in soil treated with the fungicide PCNB (pentachloronitrobenzene) varied with growing location and the level of PCNB used. Off-flavors were less evident after 3 to 4 months of storage. Information from these studies is used in developing Department recommendations for use of pesticides.



Safety of consumer procedures for roasting stuffed turkeys evaluated. Covering the breast of turkeys during roasting slowed the rate of heat penetration in this area and increased the total cooking time needed to insure destruction of any food poisoning organisms in the stuffing. Beltsville Small White and Bronze tom turkeys weighing 13 to 16 pounds and 16 to 23 pounds respectively were studied. Breast temperatures of 195° F. at end of cooking followed by a 20 minute holding period insured microbial safety of the stuffing. Frequently recommended cooking procedures giving endpoint temperatures of 185° F. in the breast or inner thigh were not always adequate to raise the stuffing temperature to 165° F. considered necessary for microbial safety. The final temperatures of the stuffing as well as that of the turkey should be considered to assure optimum doneness and safe eating.

These examples demonstrate how research in the Human Nutrition Division assists and can continue to assist the United States Department of Agriculture in its responsibility for producing enough food and a proper assortment of foods to meet the nutritional needs of the nation's citizens within the general framework of their food habits and for guiding consumers in their selection and use of foods.



## AREA NO. 1: FUNCTIONS AND METABOLISM OF NUTRIENTS

Problem. To clarify the functions and metabolic pathways of nutrients much of the nutrition research must be done with laboratory animals and lower forms of life. Only with animals of short lifespan and on controlled diets can both immediate and long-term physiological responses be measured by various biochemical, biological, and histological methods during every stage in the life cycle and during successive generations. Studies of the morphological structure, biochemical composition, and physiological function of organisms, isolated cells, and cell fragments are needed to extend understanding of nutritional processes. The kinds and amounts of nutrients and energy essential for growth and maintenance of body tissues and for nutritional well-being are influenced by such factors as climate, physical activity, and processes associated with reproduction, as well as by the hormone and enzyme activity that reflects heredity, aging, and sources of stress. Both qualitative and quantitative measures are needed of the extent to which these factors influence nutritional needs, metabolic response to various nutrient combinations, and physiological changes within tissues. Results from investigations with laboratory animals, microorganisms, and cells guide research in human nutrition and help to explain metabolic responses to diet.

### USDA AND COOPERATIVE PROGRAM

Current investigations are underway with laboratory animals to determine the effects of nutrients and foods on growth, reproduction, and longevity, on the composition of blood and tissue, and on the structure and functioning of tissues at various stages of the life cycle. Chief variables under study are the kinds and amounts of dietary fats and fatty acids, proteins and amino acids, and carbohydrates. Included also are studies of inter-relationships among nutrients when fed in purified form and when supplied from foods. Studies of cellular metabolism are developing new insights into functions, requirements, and quantitative relationships of nutrients important to the nutrition of man.

The program on the functions and metabolism of nutrients is conducted at Beltsville and under contract and cooperative agreement with private laboratories and at universities and medical schools. The studies require staff with specialized training in nutrition, biochemistry, microbiology, histology, and pathology.

The Federal scientific effort devoted to research in this area totals 22.5 professional man years distributed as follows: Lipids 9.8; proteins 6.4; carbohydrates 4.1; minerals 2.1; and vitamins 0.1.

## PROGRAM OF STATE EXPERIMENT STATIONS

Research effort in the States is concentrated on the metabolism and functions of two major classes of nutrients - fats and proteins. Small laboratory animals and microorganisms are most often used, but humans also serve as subjects. This is especially so when nutrient sources are foods which are common, acceptable, or desirable for human use. Measures and methods are constantly under investigation for refinement and suitability as tools for metabolic study.

In lipid metabolism interest focuses on type and amount of nutrient fat, cholesterol synthesis and breakdown, lipoprotein and phospholipid transport, and fat synthesis in the body. Many interrelationships are followed, such as lipid with protein, sulfur amino acids, vitamins, minerals, and hormones. When known relationships exist between these factors and vitamin-mineral dependence, they are investigated together.

Animal and plant proteins, peptides, amino acids and supplementing combinations of these serve as the dietary sources of nitrogen in metabolic studies underway. Their relative worth in meeting functional needs is compared for a given species. Comparisons are also made as to the adequacy of chosen dietary protein sources for two or more species whose metabolism differs. A large number of projects are focused on the interdependence of protein and of amino acids and other nutrients. Other studies include fats and carbohydrates, or both of them, as sources of energy, and the interdependence of minerals and vitamins. Some researches follow the synthesis of protein by experimental animals and the production of antibodies as related to amino acid intake.

Minerals under study include calcium, phosphorus, magnesium, iron, and selenium. The major activity is concerned with the role of level of dietary protein in calcium utilization, the imbalance of calcium and phosphorus in maintenance of bone structure and the part of magnesium in calcium metabolism. The relation of vitamin E and selenium when intake of unsaturated fat is high is being investigated.

There are thirty-nine States with eighty-five Federal-Grant projects focusing on unanswered problems in this area of research. Almost half of these projects represent a well coordinated effort organized under regional research authorization. This State program involves approximately 57 professional man years.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

### A. Lipids

1. Lipid - Carbohydrate Interrelationships. In research recently completed under contract at the Children's Hospital of the East Bay in Oakland, California, it was found that during the period of rapid growth the



concentration and composition of lipids in blood serum was affected to a greater extent by the kind and amount of dietary fat than by the type of dietary carbohydrate. The young puppy was chosen as the experimental animal for these studies because it had been shown that puppies reacted to diets low in linoleic acid in a manner similar to the response of young infants. Experimental diets were prepared to simulate infant formulas. All serum lipid fractions were significantly lower when very small amounts of fat rather than moderate amounts of fat were fed, irrespective of type of carbohydrate (sucrose, dextrimaltose, corn syrup, and beta lactose). Total and glyceride fatty acids were lower when a moderate amount of fat was provided by corn oil than when the same amount was provided by hydrogenated coconut oil. The lipid metabolism of puppies was little influenced by the kind of dietary carbohydrate. The only significant effect of the four dietary carbohydrates studied was on serum cholesterol levels, which were lower on sucrose diets than for other dietary carbohydrates regardless of whether corn oil or hydrogenated coconut oil was the dietary fat. All of the carbohydrates investigated, except lactose, were suitable as a source of dietary carbohydrate. With lactose, puppies grew poorly, diarrhea occurred, and mortality rate was high regardless of amount and type of dietary fat. The inability of these animals to utilize lactose was interpreted as due to lack of lactase in the intestinal mucosa, an enzyme needed for the absorption and utilization of this sugar and known to be available to the human infant. A report of this research has been accepted for publication in the July issue of the Journal of Nutrition.

2. Heated and oxidized fats. The effect of oxidation upon the nutritive value of different dietary fats has been followed in long-term studies conducted under research contracts with Columbia University, New York City, and with Swift and Company, Chicago, Illinois. Mild oxidation of olive oil, cottonseed oil, beef fat, or chicken fat had little influence on the lipid composition of the tissues when fed to rats. These fats were oxidized at 140° F. by aeration for a period of 40 hours. Continuing studies will provide information for butter and lard. The results also suggested that the body is able to rearrange the constituent fatty acids as supplied by the dietary fat and, despite similarity in total fatty acid composition, each organ appears to fashion its own triglycerides. A paper reporting these findings was presented at the meeting of the American Oil Chemist's and is being prepared for publication.

Other studies evaluated the influence of more severe oxidation produced by heating hydrogenated vegetable oil, cottonseed oil, corn oil, and lard without aeration for 120 hours at 360° F. These conditions are somewhat more drastic than would be encountered with the mild treatment that fats usually receive in the home or under the conditions of commercial use, but are still comparable to some food preparation practices. The results obtained with the fats investigated to date indicate that any changes due

to heat treatment were without harmful effects as determined by the physiological response to diets containing these fats. Animals fed the heated fats ate more than those fed unheated fats, with the greatest differences being observed in the intake of those fed lard or cottonseed oil. When fed heated or unheated hydrogenated vegetable oil, the animals lived somewhat longer than those fed cottonseed oil, corn oil, or lard. These results were reported at a meeting of the Federation of American Societies for Experimental Biology and a paper will be prepared for publication.

3. Cholesterol synthesis. The body is able to synthesize cholesterol, a sterol that is a normal constituent of body tissues. The levels of this sterol present in the tissues may be influenced by diet, and its presence in excessively high levels in the blood may be associated with degeneration of the tissues accompanied by deposition of cholesterol. The physiological effect of reducing cholesterol synthesis has been investigated in rats by feeding diets containing azasterol, which has been reported to be effective in reducing blood cholesterol levels. When diets were fed that were relatively free of cholesterol and contained this inhibitor, growth was retarded, skin abnormalities occurred, and desmosterol, an intermediate in the synthesis of cholesterol, accumulated in the blood, liver, and body lipids. The severity of the effects was influenced by the sex of the animal and by the level of corn oil in the diet with the least severe symptoms in male rats fed 30% corn oil. The addition of cholesterol to the diet delayed onset of gross symptoms in females but not in males. These studies were carried out in cooperation with the Entomology Research Division.

4. Lipid metabolism and heredity. Research has continued on the reasons for the marked variation in response to diet that is frequently observed among individuals. Biochemical measurements including cholesterol, triglycerides, phospholipids, and their fatty acid components in sera and other tissues are being used to compare the lipid metabolism of two strains of animals with different inherent characteristics and known to differ in their responses to certain dietary stresses. The cellular and subcellular structure of tissues from the same animals is being studied (see 1-A-1, 1964 report).

5. Dietary fat and insecticides. Research is being initiated that should aid in establishing whether the presence of the chlorinated hydrocarbons in food could influence metabolic response during growth and reproductive stress and periods of dietary restriction. Under contract with Swift and Company, Chicago, Illinois, investigations will be conducted to determine the effect of feeding diets containing selected types of heated and unheated fats, with and without added chlorinated hydrocarbon pesticides, on growth and reproductive performance of the rat through three generations.



The heated fats will be prepared under conditions similar to those used for a previous study (1-A-2). Selected tissues will be analyzed for pesticide residues to determine the extent to which these pesticides may accumulate in the tissues without harmful effects. The pesticides will be added in amounts that will not exceed currently accepted tolerance levels and will be in the proportions found in composite diets recently analyzed by F.D.A.

At Beltsville, the effect of the same mixture of pesticides on body chemistry and tissue structure will be investigated to determine the extent to which these pesticides may be stored in the adipose tissue of the obese rat, and to assess the physiological response when these pesticides are liberated from adipose tissue during a period of active weight loss. The diets used will contain adequate amounts of essential nutrients and have been selected on the basis of ready acceptability, resulting in heavy and obese rats when fed ad libitum. One diet is high in fat; a second contains a relatively high concentration of sucrose and moderate amounts of fat; in the third diet the carbohydrate is chiefly starch and the level of fat is low.

6. Dietary fat and serum proteins. Fatty acids are known to complex with certain protein components in blood as a means of lipid transport. Research to determine the possible effect of the kind and level of dietary fat on the relative proportion of various serum protein components has been carried out in order to obtain basic information on the response to dietary fat and to aid in our understanding of the role of the blood proteins in the transport of fat. Serum proteins were analyzed electrophoretically using moving boundary electrophoresis. Both age and kind of dietary fat were found to influence the concentration of certain of the protein components in the blood of rats. A component moving more rapidly than albumin and containing both fat and protein, was found to be particularly susceptible to the kind and level of fat in the diet as well as to age of the animal. Differences due to kind of fat were not related to any specific characteristic of the fat. The functions of these fat-protein complexes remain to be elucidated.

7. PL 480 studies of diet and fat metabolism. Many factors are known to affect fat metabolism. Among these are genetics, age, and other physiological and environmental including numerous dietary factors. In one PL 480 project in India, the effect of diet on hormone regulation of body synthesis and mobilization of fat is being studied in rats. One group of animals is receiving a fat-free laboratory diet, and others are receiving protein and fat combinations characteristic of three population diets in North, South, and Central India. For comparison other rats are maintained on regular stock ration. In another study in India, the effect of dietary proteins on fat metabolism is being studied in native monkeys in which high dietary protein had been found to increase fatty streaking on arterial walls.

In normal animals, the North India combination of 20 percent animal protein (casein) plus 20 percent butterfat caused significant rise in serum cholesterol; the South India combination of legumes (vegetable protein about 10 percent) and 10 percent coconut oil caused high initial serum cholesterol which in 30 days fell to levels below those on the stock ration; and the South India combination of another vegetable protein and 10 percent sesame oil gave the lowest serum lipid levels of all. Rats on fat-free diets of 18 percent casein and 64 percent cornstarch and 12 percent sugar had serum cholesterol significantly above animals on the stock ration or on any of the population type of diets. Maximum synthesis of body fat occurred also on the fat-free diet. Irrespective of the diet used, removal of the thyroid gland led to higher serum cholesterol but it was most exaggerated on the North India combination. Administration of thyroid hormone tended to reverse the effect on all diets. Removal of adrenal glands depressed lipid metabolism but the effect was only partially corrected by corticosteroids, one hormone produced by the adrenals.

## B. Proteins

1. Protein - Carbohydrate Interrelationships. Laboratory investigations to determine how kind and level of dietary protein and kind of dietary carbohydrate influence body composition have been completed. The results obtained are being evaluated and two papers are planned for publication. One will report the results of varying these dietary components on body composition; the second will report the effect of these dietary variables on serum cholesterol levels.

The results from investigations using a protozoan, Tetrahymena pyriformis, with nutritional requirements and metabolic responses similar to those of higher animals, indicate that this rapidly growing organism may be a useful tool for nutritional investigations of carbohydrate-nitrogen relationships. Two papers have been prepared reporting the results of these investigations to date and the findings were reported in 1964 (1-D-2). One of these papers "Free Amino Acids in Serine Antagonized Cells of Tetrahymena pyriformis" by June Wragg, Howard Reynolds, and M. L. Pelczar has been accepted for publication in the September issue of the Journal of Bacteriology. The second dealing with the effect of type of carbohydrate on L-serine antagonism is ready to submit for publication.

To obtain further information on the metabolic interrelationships among nutrients, a project has been initiated designed to investigate at the cellular level possible interactions between nitrogen and carbohydrate. For these investigations Tetrahymena pyriformis will be used to investigate the influence of varying the kind and level of carbohydrate through measurements of free amino acids and protein components in the cells, and selected enzymes active in the utilization of carbohydrates.

2. Protein and nitrogen utilization. To determine whether intact protein has a unique nutritional contribution and as a step toward using purified diets as control for investigations of the biological utilization of foods, research has been carried out to compare nitrogen utilization and energy for maintenance and body gain when the diets contain either intact protein or a mixture of purified amino acids. The ability of the rat to utilize nitrogen from the two sources investigated depended upon age, level of nitrogen and calorie intake. At the high level of calorie intake, but not at the low, and at both levels of nitrogen intake, weanling animals were able to store significantly more nitrogen from casein than from the mixture of amino acids. At the high level of nitrogen intake, adult animals receiving casein gained more nitrogen than those receiving the amino acid mixture regardless of the calorie intake but showed no significant differences when the nitrogen intake was low. The findings from this research were reported at the meeting of the Federation of American Societies for Experimental Biology in April and a paper is in preparation for publication in a technical journal.

A new project has been initiated to determine the extent of utilization of amide nitrogen and amino acids from wheat for protein biosynthesis and maintenance of tissue protein. Radioisotopes will be employed in these studies as well as the usual criteria for determining nitrogen utilization.

3. Serine synthesis. A paper is in preparation providing additional information (1964 Report, 1-B-3) concerning the formation of folic acid derivatives by Leuconostoc mesenteroides and the role of these derivatives in serine biosynthesis.

#### C. Carbohydrates

Research to determine the influence of source of dietary carbohydrate on selected enzymes in the tissues is providing further information that may help in understanding differences that occur in the physiological and biochemical response to different carbohydrates and aid in explaining the variations in response to diet among individuals. The results to date indicate that both heredity and diet may influence the concentration of some of the tissue enzymes involved in carbohydrate metabolism and fat synthesis. The diets under investigation are identical in all respects except the kind of carbohydrate. The carbohydrates include two simple sugars, glucose and sucrose, and a complex polysaccharide, cornstarch. Two strains of rats known to differ in their lipid metabolism are being used for this study. The activity of glucose-6-phosphate dehydrogenase, an enzyme involved in carbohydrate metabolism and fat synthesis, was found to be significantly higher in the livers of young animals fed sucrose than in those fed the cornstarch-containing diet. Heredity was also found to influence significantly the activity of  $\beta$ -glucuronidase and alkaline



phosphatase in the liver as well as the activity of glucose-6-phosphate dehydrogenase. The results were reported at the meeting of the Federation of American Societies for Experimental Biology in April and a paper for publication is in preparation.

#### D. Vitamins

Research on the metabolic influences of varying levels of thiamine prior to and during pregnancy has been concluded. The results have provided further information on the importance of adequate nutrition prior to as well as during pregnancy to assure the development of normal young. The findings dealing with the feeding of inadequate levels of thiamine prior to pregnancy were included in last year's report (1-C-1), and recent results provide information on low thiamine intake during pregnancy. Food intake and weight gain during pregnancy were markedly reduced when thiamine intake was low. Adequate levels of thiamine prior to pregnancy failed to permit normal growth of the mothers when dietary levels of this vitamin were low during pregnancy, but did permit better growth during pregnancy than when thiamine level was low throughout the investigation. Fetal weight was reduced significantly when thiamine levels were low during pregnancy but was uninfluenced by the thiamine level in the prepregnancy diet. Total serum protein was similar for all groups although gamma globulin concentrations were high when thiamine intake during pregnancy was low. Thiamine content in the young and in the livers of mothers was related to intake during pregnancy and was not significantly influenced by prepregnancy diet. One paper dealing with the response to low levels of thiamine prior to pregnancy has been published. A second paper, "Reproduction and Maternal Response of the Rat when Thiamine Intake is Limited", has been accepted for publication in the November issue of the Journal of Nutrition.

#### E. Minerals

A project has been initiated to determine whether wheat which has been exposed to one or to multiple fumigations with methyl bromide can cause any physiological disturbance when the wheat is a component of a diet which supplies iodine at marginal levels. The measurements to be made will include those indicative of possible metabolic disturbances that would reflect the result of a bromide-iodine imbalance on thyroid function. The Market Quality Research Division, ARS, has arranged for the purchase of the wheat to be used in this investigation and will treat, store, and analyze the wheat for fumigant residues. Wheat from the same source will be used by the Food Composition Laboratory for studies on the tocopherol and B-vitamin content of the grain and by the Food Quality and Use Laboratory for investigations on baking performance and palatability.

## F. Nutrient Interactions

Laboratory investigations to determine the nutritional value of various components of milk have been completed and will provide data on the response of rats to diets containing dried skim milk and butter oil and to milk protein with various combinations of fat, as butter oil or corn oil, and carbohydrate, as lactose or cornstarch. The results are being evaluated and will be prepared for publication.

### PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

#### General

Adams, M. 1964. Diet as a factor in length of life and in structure and composition of tissues of the rat with aging. Home Economics Research Report No. 24.

#### Lipids

Adams, M., Durand, A. M. A., and Taylor, D. D. 1964. The influence of age, dietary carbohydrate and heredity on the structure and biochemistry of the tissues of the rat. Federation Proceedings, 23, 873 (Abstract).

Poling, C. E., and Rice, E. E. 1965. Long-term nutritional responses of rats to heat-treated dietary fats. III. Final body weights, food consumption and survivals. Federation Proceedings, 24, 496 (Abstract).

Kaunitz, H., Johnson, R. E., and Miraglia, J. 1965. Composition and structure of triglycerides from dietary fats and rat organs. Journal Am. Oil Chem. Soc. 42, 132A, March (Abstract).

Ahrens, R. A., Dupont, J., and Thompson, M. J. 1965. Sterols in brain and liver of young rats fed 20,25-Diaza-Cholesterol. Proc. Soc. for Expt. Biology and Medicine 118, 436.

Dupont, J. 1965. Effects on rats of varying dietary corn oil in conjunction with feeding 20,25-Diaza-Cholesterol. Life Sciences 4, 405.

#### Proteins

Ahrens, R. A., and Womack, M. 1965. Utilization of calories and nitrogen from diets containing purified casein versus a simulated mixture of amino acids. Federation Proceedings 24, 168 (Abstract).

Carbohydrates

Chang, M. L. Wu, and Adams, M. 1965. Influence of heredity and dietary carbohydrate in some of the carbohydrate-metabolizing enzymes in the tissues of the young rat. Federation Proceedings 24, 220 (Abstract).

Vitamins

Brown, M. L., and Snodgrass, C. H. 1965. Effect of dietary level of thiamine on reproduction in the rat. Jour. Nutr. 85, 102.



AREA NO. 2: HUMAN METABOLISM AND REQUIREMENTS FOR NUTRIENTS

Problem. Research in human metabolism is conducted to determine the kinds and quantities of foods and nutrients needed by individuals for nutritional well-being, and the factors that influence nutritional needs. Systematic biochemical and physical observations of persons on self-chosen and controlled diets provide information on the use of nutrients in the body in relation to age, activity and environmental conditions; and on the quantities of nutrients and food energy required by persons of different ages, by those accustomed to different food patterns, or living under environmental and nutritional conditions. The absorption, transport, and metabolism of individual nutrients and groups of nutrients are investigated. Results of this research aid not only in defining average human requirements for nutrients and for food but also in establishing the lower and upper limits of nutrients and of food combinations conducive to human well-being. Studies also are made of the nutritional status of individuals. Such information is essential for the optimal utilization of our food resources and to all programs for improving nutrition. Application of such knowledge will influence food habits, nutritional status, market demand, and the orientation of agricultural production.

USDA AND COOPERATIVE PROGRAM

USDA research on human metabolism places major emphasis on determining the quantities of nutrients required by persons of different ages on self-chosen and on controlled diets, on measuring the metabolic behavior of individuals with regard to several nutrients at the same time, and on determining the availability and physiological utilization by man of nutrients from diets. The current program deals particularly with factors affecting the metabolism of fat and protein. Other studies seek to establish the range in biochemical response among individuals on controlled and self-chosen diets. Measurements are made on intake of nutrients, output and levels in the blood of metabolic products, and other criteria available in the living organism. Systematic compilation and reevaluation of all available knowledge on subjects of special nutritional significance are made to indicate gaps which should be filled by research, to prevent unnecessary replication of work, and to suggest the most promising areas for new research.

The program is carried out in the laboratories at the Agricultural Research Center, through contracts, grants, and cooperative agreements with universities and medical schools, and through participation in Regional Projects of the State Agricultural Experiment Stations. Nutritionists, biochemists, physiologists, physicians, and statisticians cooperate in the program.

The Federal scientific effort devoted to this research totals 7.7 professional man years distributed as follows: Nutritional requirements investigations, 1.4; food values investigations, 5.9; nutritional status investigations, 0.4.

#### PROGRAM OF STATE EXPERIMENT STATIONS

In research on the relation of nutrition to physiology two conditions are recognized; the normal, which involves the physiological functions of reproduction, lactation, growth, longevity and well-being and the stress state which may be induced by environmental and physical means including dietary restrictions. The role of nutrients both in maintaining the normal and in offsetting the stress condition is under investigation in the States. The work is based on small animal research, and many tissues are available for study. Nutritive factors which affect such conditions and are being studied include: amino acids, singly and in combination, and in conjunction with varying types of dietary fat; required energy and protein levels; adequacy of protein from natural sources; vitamins in excess and deficiency; and certain minerals as they function in relieving deficiencies.

In the area of nutrient requirements and nutritional status, the majority of the research involves human subjects. The nutrient balance in pre-adolescent children is being measured in relation to the biological utilization of protein from plant sources. This includes interrelationships of proteins with vitamins and minerals. The basal metabolic rate and the energy cost of activity is also being measured. Adolescent males, whose growth makes exceptional nutritional demands, serve as subjects for a study of physiological response to changes in dietary protein. The nutrient needs of adults include work on energy for maintenance and activity and on protein and amino acids, fats, vitamins, and minerals. A longitudinal study of adults has been underway long enough that data are beginning to emerge which may measure aging as it relates to level and variations of nutrient intake.

There are twenty-one States with forty-one projects in this phase of the human nutrition program. Eleven projects are contributing to regional studies. The program involves approximately 29 professional man years.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Nutritional Requirements

1. Preadolescents. Relatively low levels of dietary protein and relatively high levels of unsaturated fatty acids had little or no effect upon the serum lipids of 12 preadolescent girls maintained on all-vegetable diets under the Southern Regional Project S-28. For these girls (7-9 years of age), serum lipid components were essentially the same with all-vegetable diets

as with their usual mixed diets containing animal protein when the vegetable diets supplied 40 gm protein per day; when the vegetable diet supplied 22 gm protein per day, serum cholesterol and phospholipid levels remained the same but glycerides increased, possibly because the diet lower in protein contained more sugar. A manuscript presenting these results has been accepted for publication by the Journal of the American Dietetic Association.

Text and tables for a Department Bulletin, presenting detailed data on metabolic patterns of preadolescent children studied in 1954, 1956, 1957, and 1958 by seven State Agricultural Experiment Station in the Southern Region and the Division, are nearly completed.

Data obtained in this research on some 50 girls are being analyzed for nutritional and metabolic interrelationships under a cooperative agreement with the Louisiana Station.

2. Adolescents. Work is continuing under contract on a study of adolescent girls at Andrews University, Berrien Springs, Michigan. To provide information which can be used in determining the nutritional requirements of girls in this age group, the metabolic response of 16 girls, 16-19 years of age, to a controlled ovo-lacto-vegetarian type diet is being measured. Parameters on which data will be provided for each girl include nitrogen and mineral balances, intake and output of fat, blood serum lipid components (total cholesterol, total fatty acids, phospholipids, and glycerides), and blood serum riboflavin. Data reported on the first seven girls studied indicate that while they were on the controlled diet all seven girls were in positive nitrogen balance and that they digested 97% of the dietary fat.

3. Older men. Manuscripts have been prepared for publication presenting results obtained in contract research at the University of California at Los Angeles, on the effect of the type of dietary protein on the response to variations in dietary linoleic acid and at the University of Nebraska, Lincoln, and Shuman Laboratories, Battle Ground, Indiana, on the effect of the amount of dietary protein on the response to a constant amount of dietary linoleic acid. The results obtained in these studies were reported in the Multiple Use Report, page 11, for 7/1/60 to 6/30/62.

## B. Food Values

1. Amino acid patterns in food proteins. Statistical analyses of nitrogen-balance data for 24 young women fed diets containing the FAO pattern of essential amino acids and the patterns in nonfat milk solids, whole egg, oatmeal, and peanut butter were interpreted as indicating (a) that young women, when fed amino acids as in the FAO pattern, required a minimum of .22 gm tryptophan and other essential amino acids as in this pattern to maintain nitrogen balance, (b) that the nutritional value of



a protein depends in part upon factors other than the amount of the limiting amino acid, and (c) that one possible factor may have been the larger amount of nitrogen from essential amino acids in the food patterns than in the FAO pattern. The data were obtained in contract research with the University of California at Los Angeles, Oklahoma State University at Stillwater, and the University of Wisconsin at Madison. A manuscript presenting an evaluation of the combined data has been accepted for publication in the American Journal of Clinical Nutrition.

2. Evaluation of wheat. The nutritional value of wheat protein and wheat starch is under investigation under research contracts and at Beltsville. At Michigan State University of Agriculture and Applied Science at East Lansing, 12 healthy young men were maintained for 50 days on a controlled diet providing 66.6 gm protein per day of which over 90% was furnished by wheat products and the remainder by fruit and vegetables. The young men remained in good physical condition and were in positive nitrogen balance during the study indicating that wheat protein is adequate for the needs of young adult men. Most of the constituents of the blood which were included in the analyses remained within normal limits, but the level of urea dropped to about half the normal amount during the dietary period. These findings were presented at the Canadian-U.S. Conference on Nutrition, Toronto, Canada, September 1964. Additional data on amino acids and lipids will be forthcoming.

At the Agricultural and Technical College of North Carolina at Greensboro, a study was initiated to investigate the response of 13 young men to a diet with an appreciably lower protein level than the Michigan study and in which wheat provides 75% of the protein. This diet is being compared to three similar diets in which 20% of the wheat protein is replaced by protein from each of pinto beans, rice, and peanut butter. Data are to be obtained on nitrogen and mineral balance, intake and output of selected vitamins, and on the serum levels of amino acids, total cholesterol, phospholipids, glycerides, and total lipids.

At Beltsville, a study was initiated on the metabolic response of 10 young women to controlled diets in which approximately 85% of the carbohydrate is provided by either wheat starch or by sucrose. Response is being studied in terms of intake and output of nitrogen, fat, and selected minerals, blood serum protein components, blood serum enzymes, blood serum lipid levels, and the fatty acid patterns in the cholesterol, phospholipid, and glyceride fractions of the blood serum.

Research under PL 480 (Hong Kong) was initiated to study the effect on growth and other nutritional indices of children when a significant portion, about 50 percent, of the rice in the diet is replaced with wheat and when their dietary intake of selected nutrients is increased.

Approximately 270 children between the ages of 7 and 16 years were studied through one year at an orphanage in Hong Kong. Wheat in the form of bulgur and noodles replaced half the amount of rice in the diets of about 135 children and 135 other children served as controls on the usual rice diet. A mineral-vitamin supplement and a protein supplement was given to a portion of each group. Selected anthropometric and biochemical measurements of nutritional status were made of each child at the beginning of the study and 6 months and 12 months later.

Evaluation of the changes in height and weight at the end of one year indicate that there were no significant differences between the groups of children who received all rice as the chief cereal and those who received half of the cereal supply in the form of rice and half as wheat. Results of the biochemical tests and X-rays of the wrists are not yet available.

3. Metabolic response to rate of food intake. At the Beltsville laboratory, 15 young women ate a uniform diet previously described in Multiple Use Report, page 12, for 7/1/63 to 6/30/64. The rate of food intake was governed by the size and frequency of meals as served in three different dietary regimens. Statistical evaluation of the data obtained has been completed and manuscripts are being prepared for publication.

4. Carbohydrate - fat interrelationships. The interaction of dietary, genetic and hormonal factors is being studied in adult persons found susceptible to carbohydrate- or fat-induced high blood lipids in a PL 480 project in Israel. They are studied when on diets low in fat and high in starch or sugars, and when on diets low in carbohydrates and high in different types of fat.

#### C. Nutritional Status

The literature relating to the effect of limited food intake in preschool age children upon the likelihood of their reaching their physical and mental potential was reviewed and evaluated to assist in planning future research on the role of nutrition in counteracting the effects of poverty in this age group.

### PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

#### Food Values Investigations

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### AREA NO. 3: NUTRIENT VALUES OF FOODS

Problem. The nutritional value of foods to man represents the combined effects of the proportion and form of nutrients as found in single foods or as combined with others in the ordinary diet. Knowledge of these food components is essential for estimating the dietary contribution of individual foods and the nutritional adequacy of diets of population groups. Analyses of foods by chemical and physical means indicate potential nutritive value and are the basis of food composition tables used by nutritionists and clinicians. Continuing analysis of market-available foods is necessary to keep data on nutrient content current with the adoption of new varieties, and production, processing and marketing practices. Data on both cooked and raw foods are needed to determine the effects of household and institution preparation methods upon the nutrients in foods and to derive realistic figures for nutritive value of diets.

#### USDA AND COOPERATIVE PROGRAM

Foods, representative of various production sites and practices, processing and marketing procedures, are analyzed as purchased from the market and as prepared for eating by the consumer. Analyses are made for many nutrient components including amino acids, fatty acids, minerals, and vitamins. Values for calorie, protein, and fat content are derived from analyses made of the proximate composition. Methods are developed for newly identified nutrients and forms of nutrients; existing methods for known nutrients are improved and adapted for use with different foods.

The research is conducted at Beltsville, Maryland, and under contract and cooperative agreement in the laboratories of universities and industry. Chemists, biochemists, biologists, and statisticians participate in the program.

The Federal scientific effort devoted to research in this area totals 21.7 professional man-years distributed as follows: Horticultural crops, 6.4; wheat and wheat products, 8.6; animal products, 4.5; protein and amino acid values, 2.2.

#### PROGRAM OF STATE EXPERIMENT STATIONS

The work in the States on the nutritive composition of food is often concentrated on locally produced commodities. With the increase of processed and prepared foods, little of the evaluation stops with the raw product unless genetic factors and feeding and growing practices are primary objectives. The nutritive evaluation follows standard routines of processing and storage, either actual or experimentally simulated, to arrive at a value which represents the dietary contribution of the product.

The chemical structure of fats and lipids in food stuffs and the changes involved in processing, holding, and final preparation are receiving special attention as the problem of fats in human nutrition continues an active area of research and speculation. Protein and amino acid content and alteration with heat processing remain active research areas. The importance of conjugates of protein and lipids especially as they are formed in food processing, is being investigated in relation to their nutritive characteristics. Research has been directed toward the vitamin content of food as related to inherent inhibitory and stimulatory factors.

There are thirty-eight projects in this program conducted in twenty-four States. The program represents an effort of approximately 27.3 professional man years.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Horticultural Crops

1. Fruits and nuts. Analyses of fruits and nuts for content of three forms of vitamin B<sub>6</sub> have shown that most fresh fruits (including citrus, deciduous, melons, and subtropical) contained less than 1 microgram of vitamin B<sub>6</sub> per gram of fruit. Notable exceptions were avocados and bananas which had 4 and 6 micrograms per gram, respectively. Dried fruits contained 1 to 4 micrograms of vitamin B<sub>6</sub> per gram, and nuts ranged from 1 to 6.5 micrograms per gram. Coconut was found to be quite low, 0.5 microgram per gram. Generally, fruits and nuts contained a higher proportion of pyridoxine than of the other two forms of vitamin B<sub>6</sub>, namely pyridoxal and pyridoxamine. A manuscript reporting the data is in preparation.

The use of a reversed oxacetylene flame in conjunction with a "radiation" buffer mixed acid technique gave calcium results in excellent agreement with the permanganate titration procedure on a number of samples of fruits, vegetables, and composited diets. The new technique permits the use of smaller samples and allows calcium to be determined at lower levels than previously. A manuscript is in preparation.

2. Sugar extractants. Study of procedures for the extraction of sugars from fruits and vegetables is continuing. Samples of apples and carrots were used in studies to simplify procedures which have achieved some degree of acceptance through the years and to compare results obtained by different methods. The addition of calcium carbonate, refluxing the ethanol extracts, and the use of duplicate ethanol extract aliquots and duplicate colorimetric tubes were unnecessary. At least two replicate samples should be used. Differences in the two assay methods (copper and iron reduction) did not justify recommending one method over the other. The most effective ethanol concentration for extracting sugars from apples was 85% (v/v) and from carrots it was 75%.

Conditions for separating and quantitatively determining fructose, glucose, and sucrose as the trimethylsilyl derivatives and using gas-liquid chromatography are being studied.

3. Potato products. Studies are nearing completion on the proximate composition and energy value of different market forms of potato products as purchased and as prepared for serving. New ways of preparing and packaging products of this kind for the consumer give these data importance. French fries, chips, puffs, patties, and hash browned potatoes are included in these products. A manuscript reporting results of the research will be prepared.

#### B. Wheat and Wheat Products

Two new projects are focused on nutrients in wheat products. Research on the content of nutrients in a wide variety of wheats and wheat products has been initiated, extramurally under research contracts with the American Institute of Baking at Chicago, Illinois, and The Purdue Research Foundation at Lafayette, Indiana, and intramurally at Beltsville. Different varieties of wheat from the major regions of the United States where they are grown will be analyzed before and after regular and air classification milling procedures, and their baked products, which include breads by intermittent and continuous dough procedures, will also be analyzed. The study is planned to show the effects of processing on nutrients of wheat products, including study of the newer milling and baking procedures. Identical samples will be analyzed for many nutrients including thiamine, riboflavin, free and bound niacin, vitamin B<sub>6</sub> components, the individual amino acids, fatty acids, tocopherols, a number of mineral elements, and the proximate components. In addition, a wide variety of wheat products available to the consumer in 10 locations throughout the United States will be analyzed similarly. Products will include bread, cakes, doughnuts, flour, and breakfast cereals.

Preliminary studies have been made on the extraction of lipid materials from wheats and wheat flour prior to fatty acid or tocopherol analysis. Incomplete recovery of the lipids and much of the variability in the data may be minimized with improved extraction procedures. The lipid studies also included development of procedures for determining the individual tocopherols using gas-liquid chromatography. Determination of the individual forms is important because whole wheat contains several tocopherols of varying biological activity as well as an important amount of  $\alpha$ -tocopherol.

Installation of recently acquired atomic absorption equipment should enhance the determination of trace mineral elements in the wheat studies. The new equipment shows promise for yielding more reliable data on mineral elements in foods than those given by the arc spectrograph.



Under the pesticide program, studies have been initiated on possible nutritional implications of repeated use of fumigants on stored wheat. Methyl bromide, ethylene dichloride-carbon tetrachloride (3:1), and phosphine are the fumigants. The wheat will be stored for three years, with fumigation every six months. Unfumigated control samples stored at ambient and at refrigerated temperatures parallel the fumigated samples. Nutrient analyses will be made for thiamine, riboflavin, niacin, vitamin B<sub>6</sub> components, individual tocopherols, and proximate components in the wheat, the flour, and milling fractions, as well as bread and rolls baked in the laboratory. In a companion study in the Food Quality and Use Laboratory, bread and rolls and doughs from these products will be evaluated for physical properties in order to determine the effect of fumigants on the baking quality of wheat flour. The Market Quality Research Division, Stored Products and Insects Branch, and Field Crops and Animal Products Branch are cooperating in the storage, fumigation, and milling of the wheat, in determining the pesticide residues in milled flour and in making commercial baking and physical dough tests.

### C. Animal Products

1. Beef and pork. The study of changes of the fatty acid composition of the fat of ground beef and of ground pork patties due to cooking has been completed in a research contract with the University of Tennessee at Knoxville. The fatty acid composition of total lipids, neutral fat, and phospholipids was determined. A manuscript presenting the findings will be prepared.
2. Beef. Research has been initiated under contract with the University of Wisconsin at Madison, on the kinds and amounts of fatty acids in triglyceride and phospholipid fractions from fat and lean tissues of cuts of beef from the shoulder, rib, flank, and round. Analyses will be made on both raw and cooked meat. Meat from the shoulder and flank will be cooked by a moist heat method and meat from the rib and round by a dry heat method. As a part of this research biopsies of the longissimus dorsi muscle of beef animals will be made at intervals corresponding to customary market ages. The lipid portion of the muscle tissue will be analyzed for fatty acid content. This part of the study was undertaken to provide information on the effect of age of the animal at slaughter upon the fatty acid content of beef as it is available to the consumer.
3. Poultry. Research has been initiated under contract with the Food and Drug Research Laboratories, Inc. at Maspeth, New York, on possible changes in nutrient composition of eggs associated with the use of a pesticide, malathion, in management of the hens. The possible effects upon the eating quality of meat from the hens also will be evaluated. The nutrients to be studied in the eggs are the fatty acid composition of the egg lipids, vitamin A-carotene, cholesterol, and individual amino acids.

#### D. Protein and Amino Acid Values

Investigations have been initiated of enzymatic methods for complete hydrolysis of food proteins and to compare chromatographic and microbiological procedures for determining their amino acid content. The purpose of these studies is to reduce the loss of amino acids during hydrolysis. This is one of the chief sources of error in amino acid analyses of food proteins. Attention also has been given to procedures for isolation and identification of the methionine and threonine combinations which form in foods due to heat treatment and which are not utilized by the animal organism but can be made available by acid hydrolysis of the complex.

#### PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

##### Horticultural Crops

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##### Wheat and Wheat Products

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#### AREA NO. 4: FOOD PROPERTIES RELATED TO QUALITY AND CONSUMER USE

Problem. Food properties are altered by heating, chilling, freezing, aeration, physical manipulation, storage, and other practices which comprise household processes of food handling. The quality characteristics of fruits, vegetables, meats, poultry, dairy products, eggs, fats, flour, and cereals depend upon the chemical composition, physical structure, and biological systems characterizing the raw food and the changes induced by preparation procedures. Relationships should be established between the composition and structure of raw and cooked food and those qualities important to the consumer, including ease of handling, perishability, economy of yield, physical appearance, palatability and nutritive value. Such data are fundamental to developing household and institutional methods of food processing and preparation which permit optimal use of available food supplies and consumption of food for good nutrition.

#### USDA AND COOPERATIVE PROGRAM

Knowledge of the inherent chemical composition, physical properties, and biological systems in raw and processed foods is obtained to provide basic criteria for determination of those characteristics responsible for palatability and functional behavior of foods during consumer use. Principles are established and improved procedures developed for household food preparation, care, and preservation. The research is carried out along commodity lines such as fruits, vegetables, grain and dairy products, meat, poultry, and eggs. Specialized studies are also made with selected food items for use in the school lunch and other food distribution programs of the Department.

The work is conducted at Beltsville, Maryland, and through contract and cooperative agreement at private laboratories and at universities. Food specialists, chemists, histologists, and statisticians cooperate in this program.

The Federal scientific effort devoted to research in this area totals 17.3 professional man-years distributed as follows: Horticultural crops, 7.6; animal products, 7.0; oilseeds and peanuts, 1.1; cereals, 1.6.

#### PROGRAM OF THE STATE EXPERIMENT STATIONS

The preparation of food for consumer use may be accompanied by measures of quality from the raw state, through handling and processing for marketing, to final home and institutional service. In the quality work done in the States, the selected group of measures may include sensory or palatability



tests, bacterial counts or identification, chemical analysis of some labile factor, and some physical test of texture and structure. Special measures characterize certain classes of products; e.g., vitamin assays, enzymatic activity, water finding capacity, and changes in structural tissues.

The major research in product development is in the production, processing, and storage of beef, pork, lamb, poultry and eggs. A portion of the work is directed to these same factors for fruits and vegetables. Variables which affect the initial products are under study and include feeding regimens, growing practices, age, breed, and genetic factors. Conditions of processing relate to freezing temperature, duration and temperature of storage, shelf life, and the effect of light. Meat tenderness, a quality measure, is being investigated as it is affected by age and breed of animal, post-mortem aging, marbling and connective tissue.

The structure of baked products as related to the physical and chemical properties of the starches used and supplementary products involved as fats and sugars are the subjects of ongoing basic research in the carbohydrate area. The physical structure of frozen and stored batters and doughs is under study.

There is special research emphasis on the physical and chemical alterations involved in home preparation of foods. This work is carried out with the objective of having foods of maximum quality and nutritional value for final consumption. These researches include; heat penetration studies of meats of different kinds and of varying fat content; suitable methods for home freezing and storage of fresh and precooked foods; special conditions involved in high altitude cooking and baking; the effect of the use of saturated and unsaturated fats and oils on the quality of the final product baked at variable altitudes; micro-wave preparation of meats and vegetables and the chemical changes involved; and flavor characterization in frozen and stored products by means of vapor component identification.

Many of these same factors are being investigated for institutional preparation where the quantities involved impose special conditions. Heat penetration and internal temperatures of a variety of meats as related to eating quality and yield is a field of intensive study.

The program reported here includes fifty-five projects in twenty-seven States. It involves approximately 50.5 professional man years. This is a partial report of the State Experiment Station program in food science and includes the work undertaken and participated in by Departments of Home Economics. For research on food and fiber, see the reports of the Utilization Research and Development Divisions, and Clothing and Housing Division.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Horticultural Crops

1. Fruits. Investigations have been completed of the relationship between eating quality and certain acids, sugars, and other constituents of peaches, strawberries, and raspberries. Effects of ripeness, variety, season, and changes resulting from household freezing and storage were studied.

Ten varieties of freestone peaches were included over a three-year period. Four levels of ripeness were studied in the final two seasons. With increased ripeness of the fruit, there were decreases in malic and citric acids and increases in quinic acid and in pH. Sucrose content increased but glucose and fructose did not. The amount of protopectin decreased with increasing ripeness of the fruit. There were significant differences in many of the quality factors investigated that could be related to variety and season of growth. In general, the earlier maturing varieties were higher in acid content and lower in pH and sugars than were the later maturing varieties. When the fruit was frozen and stored for 8 months at 0° F. to -10° F., there were significant decreases in malic acid and quinic acid content and in pH. Sugar-acid ratios, sugars, pH, soluble solids, and citric acid-malic acid ratios were significantly correlated with panel flavor scores.

There were significant changes in composition with increased ripeness of strawberries. Citric acid, malic acid, alcohol insoluble solids, pectin, and shear force values decreased. Malic acid content decreased more rapidly than did that of citric acid. Reducing sugars, soluble solids, pH values, and panel flavor scores increased. There were significant differences in many of the quality factors that could be related to variety or season of growth. Sugar content, sugar-acid ratios, soluble solids, citric acid-malic acid ratios, and pH values were significantly correlated with panel flavor scores, and pectin and shear force values with panel texture scores. The results for peaches and strawberries have been prepared for publication and those for raspberries are being prepared.

Plans have been developed for research on the effects of the application of halogen-containing and systemic insecticides during production on the palatability, composition, and related biochemical properties of strawberries.

The effect of successive yearly herbicide treatments during crop production on the eating quality of frozen blueberries, raw apples, and applesauce was investigated. Blueberries grown in diuron- and simazine-treated soil were evaluated for flavor, pH, and soluble solids. The blueberries from plots treated with 2 lb/acre of diuron both in the spring and fall or 4 lb/acre diuron only in the spring had significantly more off-flavor

scores than those treated with 2 lb/acre diuron in the spring. The berries from the spring-fall 2 lb/acre treatment with diuron were significantly different in flavor from the control. Blueberries from the simazine treatments received a small number of off-flavor scores and were not different from the control. Herbicide treatment with diuron or simazine did not change the pH or soluble solids content of the blueberries.

Apples grown in herbicide-treated plots were significantly less tender than apples from control plots when the treatments were diuron, simazine, CIPC (isopropyl N (3-chlorophenyl) carbamate) or diuron plus CIPC, as shown by pounds force required to shear raw apple slices. Apples from amitrole-treated plots were similar to the control apples. According to panel evaluations of raw apples and applesauce, herbicide treatment did not affect flavor adversely when compared with apples grown in control plots. However, applesauce from apples grown in amitrole-treated plots received significantly more off-flavor scores than sauce made from apples grown in diuron-treated plots.

2. Vegetables. Snap beans of the Topcrop variety grown in 1964 in soil treated with the systemic insecticides Di-Syston and phorate were similar to those grown in untreated soil as shown by panel evaluations of color, flavor, and texture, and by physical measurements of color and shear (tenderness) in the cooked snap beans. In 1963, the phorate-treated beans had slightly more off-flavor and slightly lighter color than the control or Di-Syston treatment.

In other studies, preparation problems encountered in school food service units receiving dry beans distributed by USDA are being investigated. Grade 1 and Grade 3 pinto beans have been cooked in large quantity with and without sodium bicarbonate and with and without discarding the soaking water to determine cooking time, tenderness, appearance, and flavor characteristics. Other bean varieties to be investigated are great northern, red kidney, and pea beans.

Under contract with National Cannery Association Research Foundation at Berkeley, California and Washington, D. C., research was initiated to determine the extent to which pesticide residues are removed by various methods of preparation, cooking, and processing of selected vegetables, and to provide information for use in the development of effective food preparation procedures to remove or reduce pesticide residues in vegetables prepared for eating. Washing, blanching, peeling, and cooking may all be expected to modify the residue content of vegetables and to influence the amount and type of transformation products derived from the parent chemical compound of the pesticide. The vegetables chosen for the study include spinach, green beans, potatoes, and tomatoes which require different preparation and cooking procedures. These vegetables will be treated during production with selected insecticides such as DDT, malathion, and Sevin, chosen to represent a chlorinated hydrocarbon, an organic thio-



phosphate, and a carbamate-type compound with different solubilities and ease of degradation.

Plans were developed to initiate research on the effects of the use of pesticides during production of potatoes upon their palatability and related biochemical properties.

Research continued on the detection, site, and character of the lipid constituents in the edible cell walls of cantaloupes and selected fruits. Several methods have been used and adapted for staining and extracting lipids and other constituents associated with lipid-like materials in the cell walls. The findings will be prepared for publication. The above methods are being used to determine the relationship of the cell wall lipids to texture of fresh, frozen, and frozen stored cantaloupe. If indicated, the study will be extended to include other plant materials.

Investigations have been completed of the relationship between eating quality and certain acid, sugar, soluble solids, and other constituents of fresh and frozen cantaloupe. A manuscript is being prepared.

#### B. Animal Products

1. Beef. Broiled rib steaks were scored rare when broiled to an internal temperature of 140° F., medium when broiled to 160° F., and well done when broiled to 180° F. On the other hand, eye of round steaks broiled to 140° F. were medium in doneness and those broiled to 160° to 180° F. were well done. Rib steaks from more marbled carcasses contained less moisture and myoglobin and were scored higher in tenderness, juiciness, flavor, and general acceptability than steaks from less marbled carcasses; these differences were not found in steaks from the eye of round. For the most part, scores by a small expert panel for tenderness, juiciness, flavor, and general acceptability decreased as steaks (rib or eye of round) were broiled to higher internal temperatures.

Research was initiated on changes in color and tenderness of beef steaks as influenced by different rates and extents of heating by broiling. Steaks from chuck, rib, loin, and round portions of beef carcasses were broiled at temperatures of 250°, 350°, or 450° F. for different lengths of time. Changes in color, measured visually, instrumentally, and chemically, and in tenderness, measured instrumentally, will be associated with rate and extent of heating and with other factors, such as fat and moisture, that may influence rate of heat penetration.

Frozen, boneless beef roasts from mature animals, yearling, and calf, and frozen ground beef and veal have been distributed to schools participating in the National School Lunch Program. To provide information on appropriate



preparation procedures and the palatability and yield of cooked meat, roasts were cooked from the frozen state by braising and by roasting and ground meat was thawed and oven-cooked in the form of patties. The results were used as a basis for recommendations to schools.

2. Pork. A study was carried out in cooperation with the Meat Inspection Division of the Consumer and Marketing Service on the effect upon eating quality of pork sausage of the addition of antioxidants during processing. The antioxidant used was a combination of butylated hydroxyanisole and butylated hydroxytoluene. The sausages were stored under various conditions representative of marketing and household practices. The links were evaluated at intervals over a period of 16 weeks storage at 0° F. Color and odor of the raw sausage, and flavor of cooked sausage were evaluated for the freshly thawed links (24 hours at 45° F.) and after an additional four-day holding period at 45° F. The findings are being evaluated and a manuscript will be prepared.

3. Poultry. Studies of eating quality, yield, and heat penetration of defrosted turkeys stuffed and roasted to end points of 180°, 185°, and 190° F. in the thigh were completed. Increases in the end point temperature in the thigh increased cooking times and doneness and decreased juiciness. An end point of 185° F. in the inner thigh gave the best indication of optimum eating characteristics of the cooked meat. Use of hot stuffing in the colder turkeys decreased cooking times. However, this procedure extended the time the stuffing remained in the temperature zone where maximum growth of food poisoning bacteria could occur and, therefore, is not recommended as a safe practice.

Investigations were continued on the effect of cooking and end-point temperatures upon eating quality and yield of turkey roasts. Light meat turkey roasts and dark meat turkey roasts were roasted at 250°, 325°, and 400° F. to end points of 165°, 175°, 185°, and 195° F. The results have been evaluated and a manuscript is being prepared. Laboratory work also has been completed on the eating quality of both light and dark meat turkey roasts braised at oven temperatures of 250°, 300°, 350°, and 400° F. to end points of 165°, 175°, 185°, and 195° F. The data are being evaluated.

Boneless turkey roasts weighing 9-1/2 to 11-1/2 pounds each and made with light and dark meat were cooked from the frozen state for comparison with other roasts cooked after thawing. Methods of cooking included roasting at 350° F. and braising at 350° or at 450° F. to a specified internal temperature of 165° or 170° F., under conditions prevailing in school food service units. Elimination of thawing frozen roasts before cooking would be desirable in some food service units because of the possible danger from food poisoning organisms. Large boneless turkey roasts were cooked satisfactorily by any of the procedures used in this study. Frozen roasts without thawing could be roasted or braised within 4-1/3 hours and thawed roasts within 3-1/3 hours. Thawing roasts of this size required about 18 hours in a refrigerator.

Research under contract with Purdue University at Lafayette, Indiana on the microbial and quality characteristics of turkeys stuffed and roasted under different conditions was completed and a final report was submitted. A total of 230 frozen ready-to-cook tom turkeys weighing 12 to 24 pounds each were roasted at 200°, 325°, or 450° F. Dry and moist stuffings were used and turkeys were stuffed just before cooking or stuffed and refrigerated overnight. Based on results of this and other research in the Division, roasting time tables in the Home and Garden Bulletin series have been revised to reflect modern practices as well as production and processing methods currently in use. Total cooking times recommended for stuffed whole turkeys weighing from 12 to 24 pounds were reduced from 1/2 to 2 hours. The reduction in cooking time increased with the size of the turkey.

Eggs in Family Meals: A Guide for Consumers (HG 103) is the first in a new series of consumer publications being prepared. Another bulletin in preparation for the series will provide information on poultry. The new publication on eggs gives suggestions for buying and storing eggs, the principles of egg cookery, and a wide assortment of new recipes in which eggs are an essential ingredient. It also tells about selection and use of dried and frozen egg products. Each bulletin in the new series will bring together the research findings on phases of food preparation, including guides for buying food, nutritional information, up-to-date cooking methods, servings to be expected from certain amounts, and menu suggestions.

#### C. Oilseed and Peanut Flours

Research on quality and use of food products in which a major part of the protein is derived from cottonseed, peanuts, and soybeans was initiated in cooperation with the Agency for International Development and the Northern and Southern Utilization Research and Development Divisions. Basic formulas and preparation procedures are being developed for use by families and community groups in various underdeveloped countries of the world where these plant proteins are available but not used to any extent in the human diet. The flours have been used in beverages for babies, cookies, leavened and unleavened breads, stews, and other food formulations. Experimental flours prepared by new processes have been evaluated for their solubility, thickening power or viscosity, particle size, and palatability in beverages and performance in baked products.

#### D. Wheat

Research has been initiated on the effects of fumigation of wheat for insect control during storage on baking performance of flour for household use and on eating quality characteristics of bread and rolls made from these flours. Wheat samples are being stored, treated, milled, and analyzed

for fumigant residue by the Market Quality Research Division. Fumigation treatments of the wheat include: 1) methyl bromide, 2) phosphine, and 3) ethylene dichloride and carbon tetrachloride. Flour performance is being measured by oil-binding capacity, amylase activity, mixograph values, and pH. Quality of doughs is being evaluated by Kramer shear values, compressibility, recoil, and pH. Baked bread and baked roll qualities are being assessed by volume, compressibility, Kramer shear, Warner-Bratzler shear, pH, and color. Eating quality characteristics, odor, tenderness, evenness of grain, and flavor of the baked products are being scored by a trained taste panel. Measurements of flour performance and quality will be related to fumigant treatment and residues. The Food Composition Laboratory will study nutrient composition of the wheat flour and bread samples.

#### PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

##### Horticultural Crops

1964. Tomatoes on your table. Human Nutrition Research Division.  
Leaflet No. 278, 16 pp. (Rev.)

Brogdon, J. L., Dawson, E. H., Benson, A. P., and Murphy, E. F. 1965.  
The effect of pentachloronitrobenzene soil treatment on flavor of potatoes. Am. Potato Jour. 42(2): 29-36.

Sweeney, J. P., Liming, N. E., Beloian, A., and Dawson, E. H. 1965.  
Effect of household processing and storage on quality of pickled fruits and vegetables. Home Economics Research Report No. 28, 20 pp.

1965. Home canning of fruits and vegetables. Human Nutrition Research Division. Home and Garden Bulletin No. 8, 32 pp. (Rev.)

1965. Home freezing of fruits and vegetables. Human Nutrition Research Division. Home and Garden Bulletin No. 10, 48 pp. (Rev.)

Chapman, V. J., Sweeney, J. P., Martin, M. E., and Dawson, E. H. 1965.  
Fruits: Consumer quality characteristics, yield, and preparation time of various market forms. Home Economics Research Report No. 29, 28 pp.

##### Animal Products

1964. Home freezing of poultry. Human Nutrition Research Division.  
Home and Garden Bulletin No. 70, 24 pp. (Rev.)

Paul, P. C., Torton, J., and Spurlock, G. M. 1964. Eating quality of lamb. I. Effect of age; II. Effect of preslaughter nutrition; III. Overall comparisons and interrelationships. Food Technol. 18(11): 1779-1788.

- Batcher, O. M., Gilpin, G. L., Duckworth, N. R., and Finkel, P. W. 1964. Eating quality of quick-cured hams. Jour. Home Econ. 56(10): 758-762.
- Brogdon, J. L., and Dawson, E. H. 1965. Flavor evaluations of meat from Ruelene-treated animals. Jour. Econ. Entomology 58(1): 169.
- Hoke, I. M., Gilpin, G. L., and Dawson, E. H. 1965. Heat penetration, quality, and yield of turkeys roasted to an internal breast temperature of 195° F. Jour. Home Econ. 57(3): 188-191.
1965. Eggs in family meals: A guide for consumers. Human Nutrition Research Division. Home and Garden Bulletin No. 103, 32 pp.
- Gilpin, G. L., Batcher, O. M., and Deary, P. A. 1965. Influence of marbling and final internal temperature on quality characteristics of broiled rib and eye of round steaks. Food Technol. 19(5): 834-837.

#### General

- Dawson, E. H. 1964. Sensory testing guide for panel evaluation of foods and beverages. Food Technol. 18(8): 1135-1141.
1965. Freezing combination main dishes. Human Nutrition Research Division. Home and Garden Bulletin No. 40, 20 pp.



Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project: Number	Work and Line Project Titles	Work Locations During Past Year:	Line Project Incl. in	
			Summary of Progress (Yes-No)	Area and Subheading
HN 1	Nutrients and related substances in foods.			
HN 1-5C (Rev.)	Vitamin B <sub>6</sub> values in foods.	Beltsville, Md.	Yes	3-A-1
HN 1-7 (Rev.)	Proximate composition of foods as purchased and as served. **	Beltsville, Md.	Yes	3-A-2
HN 1-13C (Rev.)	Analysis of foods for fatty acid composition.	Beltsville, Md.	Yes	3-C-1
HN 1-15	Mineral elements in foods. **	Knoxville, Tenn.		
HN 1-19	Chemical determination of B-vitamins in foods. **	Beltsville, Md.	Yes	3-A-1
HN 1-20	Assay and methodological studies of sugars; and the application of these methods to the analysis of selected fruits and vegetables.	Beltsville, Md.	No	
HN 1-22	Effects of fumigation of stored wheat on vitamin content of grain, milling fractions, and home-baked products; and baking performance of flour for house- hold use. *	Beltsville, Md.	Yes	3-A-3
HN 1-23C	Nutrient composition of eggs and quality of the meat from hens treated with malathion. *	Beltsville, Md.	Yes	3-C-3
HN 1-24C	Nutrients of peanuts as affected by lindane treatment of soil. *	Maspeth, N. Y.		
HN 1-25C	Nutrient content of wheat and wheat products: amino acids, B-vitamins, and macro mineral elements. *	Beltsville, Md.	No	
HN 1-26C	Nutrient content of wheat and wheat products: fatty acids. *	Beltsville, Md.	Yes	3-B
HN 1-27	Nutrient content of wheat and wheat products: carbohydrates, trace mineral elements, tocopherols, and vitamin B <sub>6</sub> . *	Chicago, Ill.		
HN 1-28C	Fatty acids of fat and lean portions of uncooked and cooked beef and pork. *	Beltsville, Md.	Yes	3-B
HN 2	Functions of nutrients and their metabolic interrelationships.	Lafayette, Ind.		
HN 2-15	Analysis of records of a rat colony and development of animals for specific experimental purposes.	Beltsville, Md.	Yes	3-B
HN 2-24	The effect on rat carcass composition of varying proportions of dietary proteins and carbohydrates.	Beltsville, Md.	No	
HN 2-35	Effect of graded levels of dietary thiamine prior to and during pregnancy on reproduction in the rat. **	Beltsville, Md.	Yes	1-B-1
HN 2-36	The influence of diet on the sequence of histological changes in two strains of rats.	Beltsville, Md.	Yes	1-D
HN 2-37	The influence of age and diet on enzyme systems in selected tissues of two strains of rats.	Beltsville, Md.	Yes	1-A-4
HN 2-38	Influence of kind of dietary fat and carbohydrate on the chemistry and histology of the tissues at different stages in the life cycle of the rat.	Beltsville, Md.	Yes	1-C
HN 2-39	Exploratory investigations on the effect of diet on body composition of the rat, as determined in vivo or by carcass analyses.	Beltsville, Md.	Yes	1-A-6
		Beltsville, Md.	No	

\* Initiated during reporting year.

\*\* Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project:			Line Project	Incl. in
Number	Work and Line Project Titles	Work Locations During Past Year:	Summary of Progress (Yes-No)	Area and Subheading
HN 2-40	The influence of diet on the lipid metabolism of two strains of rats at various stages in their life cycle. *	Beltsville, Md.	Yes	1-A-4
HN 2-41	The metabolic response of the rat to diets containing high levels of bromide residues. *	Beltsville, Md.	Yes	1-E
HN 2-42	Utilization of amino acid and amide nitrogen of wheat in tissue protein maintenance. *	Beltsville, Md.	Yes	1-B-2
HN 2-43C 2/	The physiological response of rats to diets which include different kinds of fats with and without added hydrocarbon pesticides. *	Chicago, Ill.	Yes	1-A-5
HN 2-44	The metabolic effects of pesticide residues in body fat when the content and distribution of body fat of rats fed different diets are altered by dietary restriction. *	Beltsville, Md.	Yes	1-A-5
HN 2-46	Metabolic relationships between carbohydrate and nitrogen. *	Beltsville, Md.	Yes	1-B-1
HN 2-48	Utilization of dietary protein as affected by in vivo breakdown of urea. **	Chicago, Ill.	No	
HN P-1	Pioneering Laboratory for Cellular Metabolism. **	Beltsville, Md.	Yes	1-B-1,3
HN 5	Biological evaluation of foods and diets. **			
HN 5-3C	Lipid metabolism during growth as affected by kind and amount of dietary fat and carbohydrate. **	Oakland, Calif.	Yes	1-A-1
HN 5-4C	Nutritional response of rats to diets containing selected types of heat-treated and rancidified fats.	Chicago, Ill.	Yes	1-A-2
HN 5-6	Nutritional value of various components of milk when fed singly and in combination to rats.	New York, N. Y.		
HN 5-8	Lipid biosynthesis as a criterion for assessing the biological value of foods.	Beltsville, Md.	Yes	1-F
HN 5-9	Utilization of energy from diets containing purified protein versus a simulated mixture of amino acids.	Beltsville, Md.	Yes	1-A-3
HN 3	Food quality, preparation, and preservation.			
HN 3-8	Quality evaluation of selected foods and food products exposed to agricultural chemicals. **	Beltsville, Md.	Yes	4-A-2; B-2
HN 3-19	Physical, chemical, and eating quality characteristics of fruits in relation to household practices. **	Beltsville, Md.	Yes	4-A-1,2
HN 3-21C 1/	Preparation factors influencing the quality characteristics of cooked poultry meat.	Beltsville, Md.	Yes	4-B-3
HN 3-22	Investigations of the constituents in cells and cell walls of fruits as related to eating qualities.	Beltsville, Md.	Yes	4-A-2
HN 3-24	Changes in color and tenderness of beef as influenced by different rates and extents of heating by broiling. *	Beltsville, Md.	Yes	4-B-1

\* Initiated during reporting year.

\*\* Discontinued during reporting year.

1/ Supported in part by funds from Consumer and Marketing Service.

2/ Supported in part by funds from SURD.

\* Initiated during reporting year.  
 \*\* Discontinued during reporting year.  
 1/ Supported in part by funds from Consumer and Marketing Service.  
 2/ Participating Agency Service Agreement with the Agency for International Development.  
 3/ In cooperation with Southern Regional Project S-28, revised.

PL 480 Research Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project: Number	Work and Line Project Titles	Work Locations During Past Year:	Line Project Incl. in	
			Summary of Progress (Yes-No)	Area and Subheading
PL 480				
A6-HN-1	:Studies on the nutritive values of protein; : and availability of amino acids to human : subjects on a low protein diet.	:Taipei, Taiwan	: No	
A6-HN-2	:Nutritional studies on rice and sweet- : potato supplementation for the : improvement of Formosan diet.	:Taipei, Taiwan	: No	
A7-HN-4	:Metabolism of ascorbic acid.	:Calcutta, India	: No	
A7-HN-5	:Biochemical and nutritional studies of : leaf proteins.	:Calcutta, India	: No	
A7-HN-6	:Effects of protein malnutrition and of : different food sources of protein on : learning performance.	:Baroda, India	: No	
A7-HN-8	:Studies of hormonal regulation of : cholesterol and fat metabolism.	:Calcutta, India	: Yes	1-A-7
A7-HN-10	:Ascorbic acid secretion during lactation.	:Baroda, India	: No	
A7-HN-14	:The influence of quality and quantity of : dietary proteins on the lipid : metabolism. *	:Delhi, India	: No	
A7-HN-15	:Protein nutrition and lipid metabolism : in Rhesus monkeys. *	:New Delhi, : India	: Yes	1-A-7
A7-HN-17	:Iodine requirements and iodine storage : in India. *	:New Delhi, : India	: No	
A10-HN-1	:Development and biological evaluation : of protein in mixtures of foods : formulated from vegetable sources. **	:Jerusalem, : Israel	: No	
A10-HN-2	:Nutritional studies of carbohydrate- : and fat-induced lipemias.	:Jerusalem, : Israel	: Yes	2-B-4
A10-HN-3	:Studies on ultrastructural changes in : essential fatty acid deficiency. *	:Jerusalem, : Israel	: No	
A10-HN-4	:Nutritional evaluation on infants of a : protein mixture from vegetable sources. *	:Jerusalem, : Israel	: No	
A11-HN-1	:Nutritive value of "tempeh". *	:Osake, Japan	: No	
A13-HN-1	:The studies of basal metabolism and : energy expenditures of Koreans in daily : life and work.	:Seoul, Korea	: No	
A25-HN-1 (FAS)	:Growth and other nutritional responses : of children to increased intakes of : selected nutrients. **	:Hong Kong, : China	: Yes	2-B-2
E15-HN-2	:Food additives and lipid metabolism.	:Milan, Italy	: No	
E21-HN-1	:Study on availability and mechanism of : carotene and vitamin A utilization : from different dietary sources and under : different experimental conditions.	:Warsaw, : Poland	: No	
F4-HN-1	:A survey of amino acid content and : biological value of the protein in major : varieties of food crops in Egypt. **	:Cairo, Egypt : U.A.R.	: No	

\* Initiated during reporting year.

\*\* Discontinued during reporting year.





